

**Screening of secreted proteins of *Sporisorium reilianum* f. sp. *zeae* for cell death suppression in *Nicotiana benthamiana***

**Deiziane Dutra<sup>1</sup>, Nisha Agrawal<sup>1,4</sup>, Hassan Ghareeb<sup>2,3,\*</sup>, and Jan Schirawski<sup>1,3,4,§</sup>**

<sup>1</sup> Microbial Genetics, Institute of Applied Microbiology, RWTH Aachen University, Aachen, Germany

<sup>2</sup> Plant Biotechnology, National Research Centre, 12311 Cairo, Egypt

<sup>3</sup> Molecular Biology of Plant-Microbe Interactions, Albrecht-von-Haller Institute of Plant Sciences, Schwann-Schleiden Research Center, Georg-August-University Göttingen, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

<sup>4</sup> Genetics, Matthias-Schleiden-Institute, Friedrich-Schiller-University Jena, Philosophenweg 12, 07743 Jena, Germany

\* Present Address: Plant Cell Biology, Albrecht-von-Haller Institute of Plant Sciences, Georg-August-University Göttingen, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

**§ Author for correspondence:**

Jan Schirawski

[jan.schirawski@uni-jena.de](mailto:jan.schirawski@uni-jena.de)

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**Table S1. Oligonucleotides used in this study.** Oligonucleotides used for cloning contain the attachment sites (*attB1* for forward primer and *attB2* for reverse primer) necessary for Gateway cloning. Oligonucleotides used for Gibson assembly contain a 20 bp overhang.

Gene of interest	Plasmid construct	Primer name	Primers for cloning
sr10069	pHG44-GWY_sr10069	oKC3	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGTGCACCTCACCTCG
		oKC4	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTATGGCTCAACGATGCG TAG
sr10077	pHG44-GWY_sr10077	oKC5	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAATCTTCTGCCCTTCAAAC
		oKC6	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTATACTGATAAATGGAG AGCAGG
sr10529	pHG44-GWY_sr10529	oKC9	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCTGGTCCACTCG
		oKC10	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAGTGGCTCTTGTACCC
sr13458	pHG44-GWY_sr13458	oKC11	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGCTCACCGTATTCAAGTTCG
		oKC12	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGGCTGCAGAGGCAGG CATC
sr13524	pHG44-GWY_sr13524	oKC13	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGATCCAAACCGCCCTG
		oKC14	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTACTTCCACACATAGTC CG
sr16441	pHG44-GWY_sr16441	oKC21	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGGTTCTCGGTCAGCTC
		oKC22	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGCGGCCCAAAGGTT TATC
sr11947	pHG44-GWY_sr11947	oKC23	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGCTCCTCGCATCCTTCG
		oKC24	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCACACCAGACCTTGTTG GCTAGAG
sr13419	pHG44-GWY_sr13419	oKC27	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGGTCCACTCTCCACCTCTTC

sr11402	pHG44- GWY_sr11402	oKC28	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTACCATGCGAGATTGCT CGTGATCC
		oKC29	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGATGCACAGCGGC
		oKC30	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAGTCTTCCACAGCAC GTC
sr14168	pHG44- GWY_sr14168	oKC15_ new	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCCT TGCCAACCTCGAAGCTAC
		oKC16_ new	GGGGACCACTTTGTACAAGAAAGC TGGGTGTGCGAGCGATCAAAGAGAC AG
sr11400	pHG44- GWY_sr11400	oKC31	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGATCAACAACGCCCTCC
		oKC32	GGGGACCACTTTGTACAAGAAAGC TGGGTGTGAGCACTTGACCAGGTC ATGCTC
sr15147	pHG44- GWY_sr15147	oKC33	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGAACTCATTCTTTCTCGTGTC
		oKC34	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGATGATCTGAGGGGC AGGG
sr16247	pHG44- GWY_sr16247	oKC41	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCTTACCAAGTACGCCGTCTC
		oKC42	GGGGACCACTTTGTACAAGAAAGC TGGGTGTGAGGCTGAGTAGATGCG GTCTGC
sr12084	pHG44- GWY_sr12084	oKC45	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGTTCAGCTCAAGTCGTCGTTC
		oKC46	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGGGCTCGTCGGCCTT CTTGG
sr10767	pHG44- GWY_sr10767	oKC51	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGTCTTCATTCTTGCTCCC
		oKC52	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCACTGTCTTGCCGGACG
sr16553	pHG44- GWY_sr16553	oKC53	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGATTGCAACTTTGACGGTCTT G
		oKC54	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGGCAACATTTCTGGC TTGCTTAG

sr17149	pHG44- GWY_sr17149	oKC55	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGAAGCCTCAACTTTATC
		oKC56	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAAATCCAGATCGCTCT C
sr16558	pHG44- GWY_sr16558	oKC59	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAACAATTTTCGTCTGATGGCCCT GGCG
		oKC60	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGCCACGCATCCTTTG AATGACG
sr13420	pHG44- GWY_sr13420	oKC61	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCCGACATACGTCGACAC
		oKC62	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCATTGTAAAGGAAGAGG ATGG
sr14941	pHG44- GWY_sr14941	oKC63	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTTGGTGCGTTTCACAACGCTTGC
		oKC64	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAGGGCTTGAATTTCTC ACGTACTC
sr13897	pHG44- GWY_sr13897	oKC64	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAGGGCTTGAATTTCTC ACGTACTC
		oKC72	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAGGCAGAGTCTGCAAT GG
sr13903	pHG44- GWY_sr13903	oKC77	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCCC GTCTTCACCAACAATGTCTC
		oKC78	GGGGACCACTTTGTACAAGAAAGC TGGGTGGCGCTAAGTGCTGCAGTC TC
sr13905	pHG44- GWY_sr13905	oKC79	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAG CGCAACTCTGCAACAACAGC
		oKC80	GGGGACCACTTTGTACAAGAAAGC TGGGTGAAGCAGAGCCTAAGCAGA GAAG
sr14274	pHG44- GWY_sr14274	oKC89	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCCT GGCAAGTCTTTCAAGTCGACAC
		oKC90	GGGGACCACTTTGTACAAGAAAGC TGGGTGACGAGCAAGCCGTTCTGA GTACC

sr11006	pHG44- GWY_sr11006	oKC107	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAG GCCTAGACACCTTTAGCACAAC
		oKC108	GGGGACCACTTTGTACAAGAAAGC TGGGTGGATCACCAACGATCTGGC TACTC
sr11002.2	pHG44- GWY_sr11002.2	oKC99	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGTTCAACCTTCCACATCC
		oKC100	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTTACTACCACCCGTAGT GGTTATG
sr14226	pHG44- GWY_sr14226	oKC101	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAA TGTCGCCTTTCCACCTTCTC
		oKC102	GGGGACCACTTTGTACAAGAAAGC TGGGTGCACGCCTTACACCTAAAC ATGC
sr14685	pHG44- GWY_sr14685	oKC103	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCCC AATCATGCTCTCGCATCTACC
		oKC104	GGGGACCACTTTGTACAAGAAAGC TGGGTGGAGCGTCTAGGCCAGATA CTCG
sr02614	pHG44- GWY_sr02614	oNR131	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTCGACCACTCAGCG
		oNR132	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAGTTGGCCTTGGGCTTC TCTTTCC
sr11130	pHG44- GWY_sr11130	oNR75	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCTGATGTCAAACCGATCG
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sr11133	pHG44- GWY_sr11133	oNR121	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTCGACCCAGAAGCC
		oNR122	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTACTTCTTCTTCTTGACC
sr13367	pHG44- GWY_sr13367	oKC19	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTTGGTAACAGCTATCATGC
		oKC20	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTATTTTGGCCCTTGGGA AGC
sr13374	pHG44- GWY_sr13374	oNR133	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTCTGTCTCGACCACGAAGC

sr13864	pHG44- GWY_sr13864	oNR134	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAGCGCGGGCATGGG
		oNR113	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCTGTCAAAGCCGACC
		oNR114	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAAACTGGAAAGCCAG C
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		oNR156	GGGGACCACTTTGTACAAGAAAGC TGGGTGTACGTTGCTCCCGACTTT G
sr14220	pHG44- GWY_sr14220	oNR163	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCTCTTCAAGGCTCAAGC
		oNR164	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAATCGGTATCACCATC ACTTTC
sr16561	pHG44- GWY_sr16561	oNR91	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCAGATTCAGCGACTCGTCACC
		oNR92	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCATACCGCTTCGCGCAC AACC
sr17138	pHG44- GWY_sr17138	oNR93	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGTGCTACCTCGTCGTTGTCG
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sr20006	pHG44- GWY_sr20006	oNR135	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGTTCTTCCAAATCCTCATTGC
		oNR136	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAGAACCAGCCTGAGCT CG
sr14222	pHG44- GWY_sr14222	oNR151	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTTTCAGCCGCAACAAGTCC
		oNR152	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAGATACCACGGCCGTC GTC
sr11355	pHG44- GWY_sr11355	oNR127	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGCTCAACGCCTAC
		oNR128	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTACCAGAACCAGCTGAC C

sr11132	pHG44- GWY_sr11132	oNR77	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GATGGCCACTCAATTAGCTGTG
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sr12085	pHG44- GWY_sr12085	oNR123	GGGGACAAGTTTGTACAAAAAAGC AGGGGACAAGTTTGTACAAAAAAG CACATGTTCAAGCCCTC
		oNR124	GGGGACCACTTTGTACAAGAAAGC TGGGTCTCAAGGGGTATGTGATCT CT
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		oNR120	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAGGGATGGGCCTTTGA C
sr13904	pHG44- GWY_sr13904	oNR159	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GACATCTTTAGGATTTGCTCGGG
		oNR160	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTATCGGCCTTGTTGACCC
sr14221	pHG44- GWY_sr14221	oKC83	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCGC AGCGATGATCGCTCAAAC
		oKC84	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAGGCCTCTTACTAGG CTGTC
sr10702	pHG44- GWY_sr10702	oNR177	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGTCGAGCTTCGTCG
		oNR178	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCACTTCTGGCACTTGTTCC C
sr12897	pHG44- GWY_sr12897	oDD_52	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GAAGTTCGCCTTCGGC
		oDD_53	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAACCCGAGGTGTTGAT GTC
sr10314	pHG44- GWY_sr10314	oKC7	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCGCTTCGACGGAACCTTG
		oKC8	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAACGTTCTGAAGGTGC ATGAC
sr10532	pHG44- GWY_sr10532	oKC65	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCGAACCTCTCCCGCTCTG

sr17609	pHG44- GWY_sr17609	oKC66	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTAAGCCGAGTCCACGTC AG
		oKC81	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCCA CCCAAACATCCATTTACG
		oKC82	GGGGACCACTTTGTACAAGAAAGC TGGGTGGTCGCTTTGCGTGGAGAT TC
sr12538	pHG44- GWY_sr12538	oKC95	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCGA CCCAATCCAGCAAGTCAGAG
		oKC96	GGGGACCACTTTGTACAAGAAAGC TGGGTGAGCGAGCTTGCGACATC AG
sr11238Δsp	pHG44- GWY_sr11238Δsp	oBF9	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCCCCACCGGTGATCAAG
		oNR168	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCAAGGCTTACGGATCTT GC
sr16441Δsp	pHG44- GWY_sr16441Δsp	oBF26	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCGCCCATGTTCAAGTACC
		oKC22	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGCGGCCCAAAGGTT TATC
sr13367Δsp	pHG44- GWY_sr13367Δsp	oBF13	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCTCCCATGTGGAACAAC
		oKC20	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTATTTTGGCCCTTGGA AGCTC
sr13458Δsp	pHG44- GWY_sr13458Δsp	oBF18	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGTGCTCAAGTTTGGTGCTGGCAT C
		oKC12	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGGCTGCAGAGGCAGG CATC
sr14387Δsp	pHG44- GWY_sr14387Δsp	oBF24	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GCCTTTGGTACCTGGAAGCTTCG
		oKC36	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTACTGACCACGGCCCCA AAG
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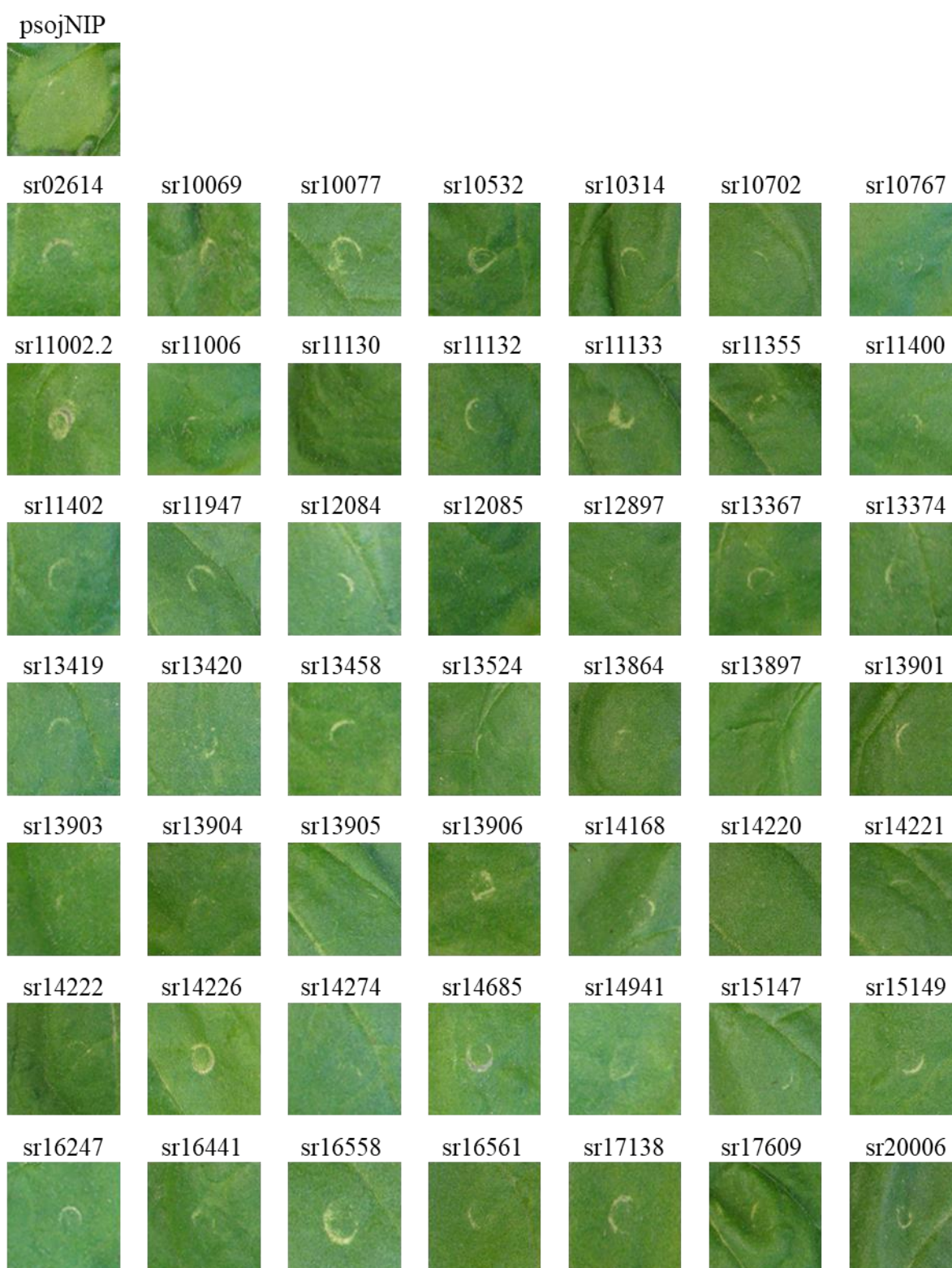


sr16553Δsp	pHG44-GWY_sr16553Δsp	oKC44	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGTCCTTCTGCTTTCCA CTGCCG
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		oKC54	GGGGACCACTTTGTACAAGAAAGC TGGGTGCTAGGCAACATTTCTGGC TTGCTTAG
sr10767Δsp	pHG44-GWY_sr10767Δsp	oBF8	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGCCCCAAGAGGAGCCCTCTTC
		oKC52	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCCTGTCTTGCCGGACG
sr13420Δsp	pHG44-GWY_sr13420Δsp	oBF17	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGAGACACCTCTAGAGGGAAGAG
		oKC62	GGGGACCACTTTGTACAAGAAAGC TGGGTGTCATTGTAAAGGAAGAGG ATGG
sr10057Δsp	pHG44-GWY_sr10057Δsp	oBF1	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GTGGGGAAAACATCCAAAGTATC
		oKC2	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTACAGAGGAAACCGGGG GAAG
GFP	pHG44_GWY_GFP	oNR103	GGGGACAAGTTTGTACAAAAAAGC AGGCTTCGAAGGAGATAGAACCAT GGTGAGCAAGGGCGAGG
		oNR104	GGGGACCACTTTGTACAAGAAAGC TGGGTGTTACTTGTACAGCTCGTCC
INF1	pHG44_GWY_INF1	oDD_70	GGGGACAAGTTTGTACAAAAAAGC AGGCTCAATGGGATTTGTTCTCTTT TCAC
		oDD_24	GGGGACCACTTTGTACAAGAAAGC TGGGTATCATAGCGACGCACACGT AGAC
Amplifi- cation	Description	Primer name	Primers for Gibson assembly
pHG44 backbone	Forward	oDD_05	CTGTGTGAAATTGTTATCCGTGAC AGGATATATTGGCGGGTAAAC
pHG44 backbone	Reverse	oDD_06	TACATTCAAATATGTATCCGCGTCC GCAATGTGTTATTAAGTTG
cDNA Amplifi- cation	Description	Primer name	Primers for expression analysis
sr14220	Forward	oNA226	GAAGTATGCCAACGCTACTG
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sr14226	Forward	oNA238	CATCCTGCGTACATCAGCC

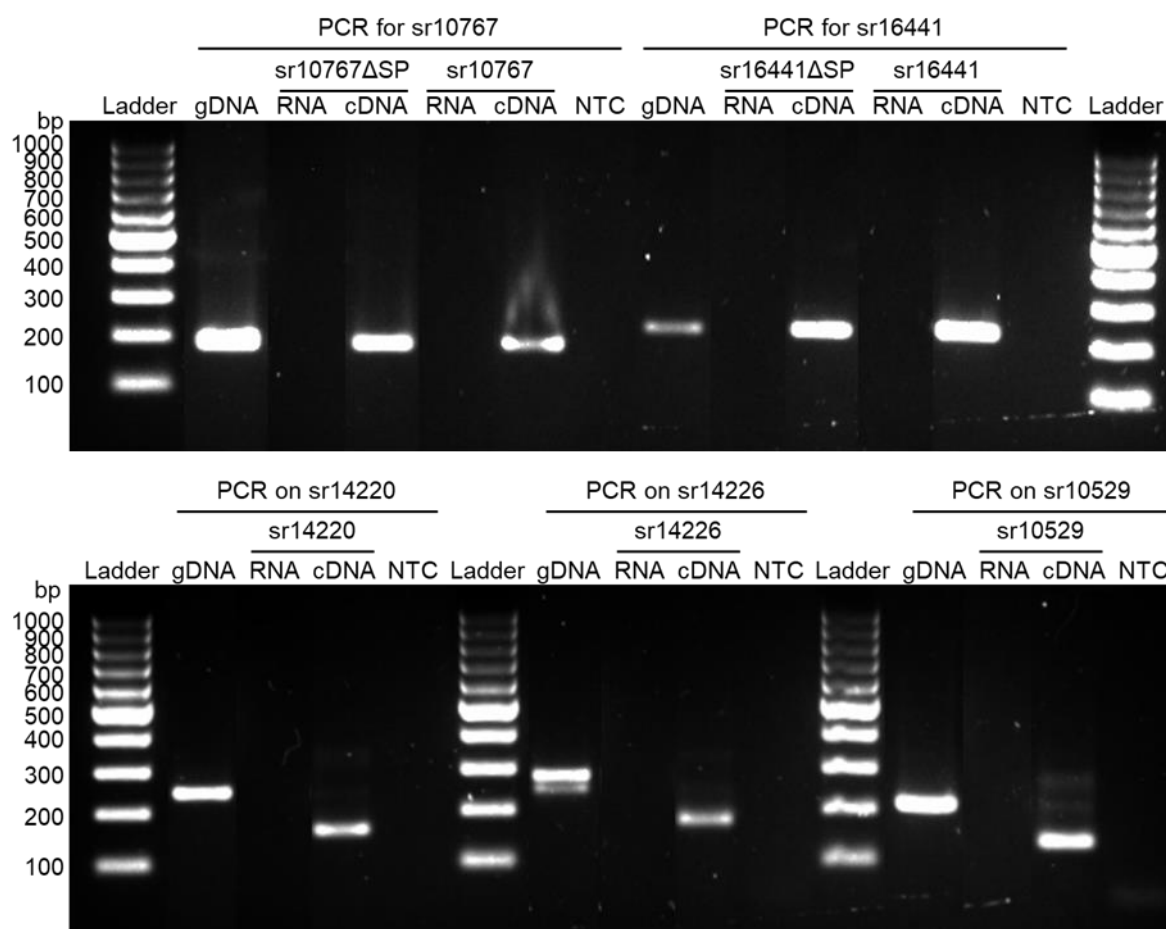
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	Forward	oNA270	GCCTCGTGCTCCATGTTCA
sr16441Δsp and sr16441	Reverse	oNA233	GCCTCCGAAGTCGAAGAAC
	Forward	oNA230	CGTCCAAGATTCGCACATTC
sr10767Δsp and sr10767	Reverse	oNA231	GATCTGGTCAGAGGTCAATG
	Forward	oNA228	GAGGAAGCTGCCAAGGATG
	Reverse	oNA229	TCGTTCGAGTCGTCTGAAG

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## Supplemental Figures

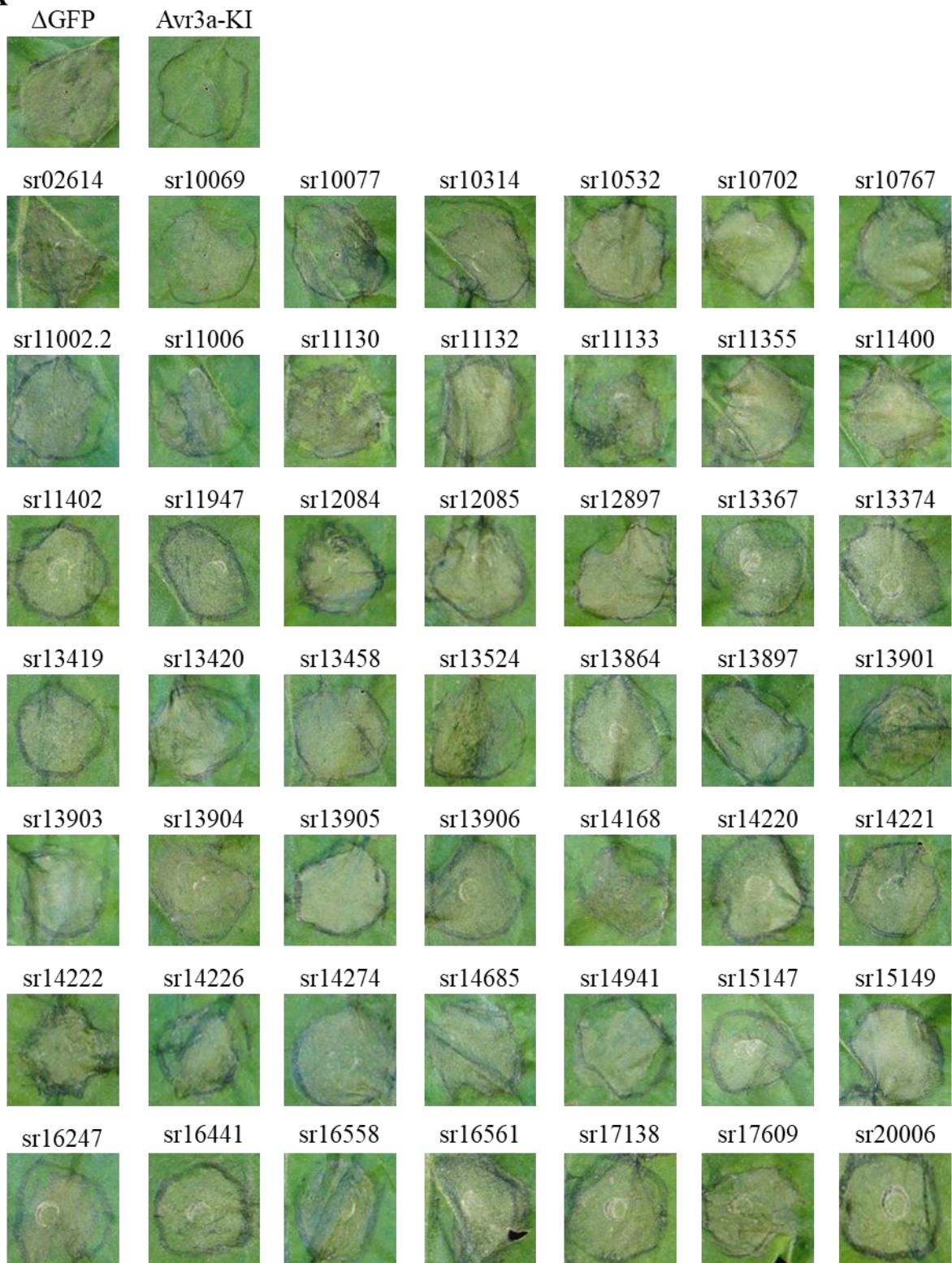


**Figure S1. PCD induction assay.** The assay was conducted at TSL, UK. *Agrobacterium* strains carrying constructs with SRZ effectors were infiltrated in *N. benthamiana* along with the control psojNIP and evaluated 3-5 days later. None of the constructs could induce cell death under the tested conditions. The picture was taken at 4 days after infiltration.

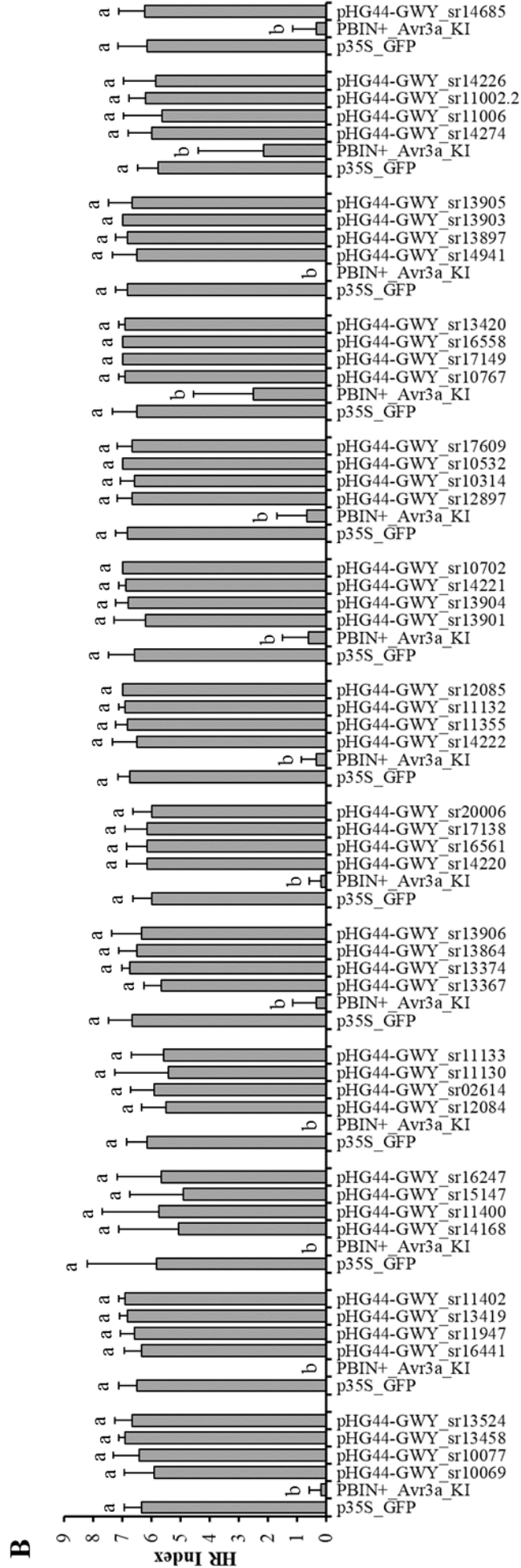


**Figure S2. Expression and demonstration of splicing of selected candidate effectors in *N. benthamiana* as tested by RT-PCR.** *N. benthamiana* leaves were infiltrated with *A. tumefaciens* transferring gene expression constructs for the indicated candidate effectors. At 4 dpi, total RNA was extracted, treated with DNase, and used for cDNA generation. PCR was done on RNA after DNase treatment (RNA), on cDNA (cDNA) and on genomic DNA of *S. reilianum* (gDNA) as positive control. The no template control (NTC) contained water instead of template. The samples were run on a 1.7% TAE agarose gel. The PCRs on RNA and NTCs do not show products. PCR products of gDNA have expected sizes of 187 bp (sr10767 and sr10767ΔSP), 226 bp (sr16441 and sr16441ΔSP), 283 bp (sr14220), 237 bp (sr14226), and 210 bp (sr10529). PCRs on cDNA have expected sizes of 187 bp (sr10767 and sr10767ΔSP), 226 bp (sr16441 and sr16441ΔSP), 156 bp (sr14220), 168 bp (sr14226), and 128 bp (sr10529). The samples of the upper and lower panels were run on the same gel, respectively, photographed, and the picture was cut and reassembled for easier labeling of the lanes.

**A**







**Figure S3. PCD suppression assay.** The assay was conducted at TSL, UK. *Agrobacterium* strains carrying constructs with SRZ effectors were infiltrated in *N. benthamiana* along with GFP (negative control) and Avr3a-KI (positive control), one day later the infiltration sites were challenged with the elicitor INF1. The evaluation was done from 3-5 days after infiltration. (A) None of the 49 constructs tested in this experiment could suppress INF1-induced cell death (B) Quantitative comparison of necrosis induced by INF1 in the presence of PCD suppression candidates show no PCD suppression by SRZ effectors. The columns shows the mean and standard deviation. The letters above each column indicate statistically significant differences of the HR index ( $P < 0.01$ ).